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REVIEW
ОБЗОРНАЯ СТАТЬЯ

Silver nanoparticles: known and unknown facts in a review of properties, mechanisms of action, and medical applications

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Abstract. *Relevance.* The review focuses on the current state of the art in the use of nano-objects in medicine and pharmacy. The relevance of this topic stems from the unique properties of nanomaterials, which exhibit various size-dependent properties in the range of 1–100 nm, where quantum phenomena come into play. The *aim* of this work is to outline the main strategies of modern science for the development and successful stabilization of nanoparticles intended for both the diagnosis and treatment of pathological diseases. Nanostructured heterogeneous matrices containing highly dispersed particles with sizes corresponding to the de Broglie wavelength open up promising opportunities for their use in various scientific and clinical studies. The diversity of the nature, morphology, sizes, and properties of nanoparticles used in medicine for the diagnosis and treatment of diseases of bacterial, viral, and malignant neoplasm stimulates interest in particles exhibiting quantum size effects (QZE). The QZE of nanoobjects are characterized by unique optical and magnetic properties that differ from the properties of physical structures in which particles are larger than 100 nm. Fluorophores based on quantum heterogeneous structures are actively used in medicine and biology, for example, in systems for labelling and visualizing biological data, including in the detection and control of tumor cells. *Conclusion.* This review describes modern approaches to the synthesis and functionalization of nanoparticles based on top-down and bottom-up principles, as well as the potential mechanisms of action of nanoparticles depending on their morphology, size, and ionic (atomic) form. The unique physicochemical properties of nanoparticles, which are due to quantum size effects, are discussed. The article describes various forms of nanoparticles, mechanisms of action, molecular targets, as well as methods of synthesis and application. These aspects are crucial for understanding the role of nanoparticles in combating infections and developing new therapeutic approaches, ensuring their continued relevance in modern science and medicine.

Keywords: silver nanoparticles, biocidal properties, antimicrobial surfaces, quantum size effect, oligodynamic effect, green chemistry

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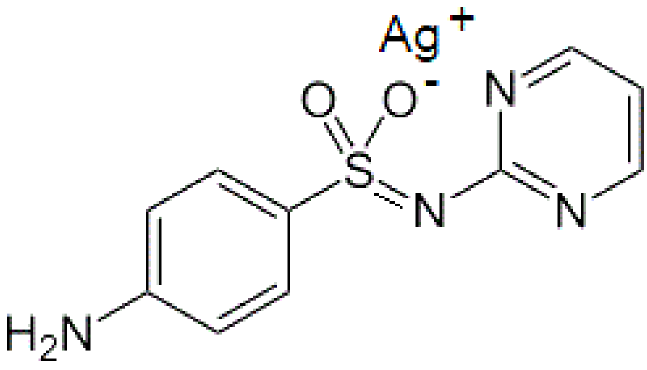
Introduction

For a long time, silver and its compounds have been widely used in various fields of human activity, including medicine and everyday life, due to their pronounced biocidal properties due to the high reactivity of the metal [1]. The use of silver for the treatment of various diseases or to prevent the transmission of infection dates back to 4,000 years BC. In the manuscripts, there were mentions of a dangerous biogenic agent that threatens human health in enclosed spaces — mold associated with the so-called “leprosy” [2]. This disease served as a generalized term for a group of infectious dermatological diseases caused by bacteria or fungal spores, which were treated with metals and their derivatives. Silver and its compounds have been used to fight various microorganisms since the time of ancient civilizations. For example, the ancient Greeks and Egyptians used silver vessels to store drinking water and other liquids, which significantly reduced the risk of infection. In the Middle Ages, the well-to-do sections of the population ate from silverware, which is believed to have prevented the reproduction of pathogenic microbes. According to some theories, such dishes could protect nobles from the devastating effects of the bubonic plague pandemic [3].

The active introduction of silver compounds into medical practice began with the use of silver nitrate, which was originally used as a solid mass, known as "lapis lazuli" (from lat. *lapis* — stone and *lazur* — blue): mineral from the class of silicates with a framework structure of crystal grid, chemical composition $Ca_2Na_6[AlSiO_4]_6[SO_4][S_2]$. Silver nitrate was used throughout the 1800s to treat burns, ulcers, and infected wounds [4]. The use of silver, according to official European pharmaceutical practice, dates back to 1881 with the experience of the German obstetrician-gynecologist Karl Kred, who proposed a method for the prevention and treatment of neonatal inclusion blennorrhea in newborns by instilling 1–2% silver nitrate solution into the eyes [5]. Since the beginning of the 19th century, silver nitrate solutions have become even more widespread in medicine: at the beginning of the 19th century, a dosage form was proposed that included a 3.4% solution of silver nitrate with the addition of linseed oil, which was used to treat pathologies associated with excessive overgrowth of connective tissues due to inflammation. This formulation has proven itself to be the standard for preoperative preparation, although its use declined after World War II and the advent of antibiotics. This discovery stimulated doctors, especially surgeons, to search for new effective ways to use silver nitrate. One of the key areas was the treatment of wound injuries and related infectious complications. Biochemist Charles Lewis Fox (USA) revived the use of silver compounds by developing in 1966 an ointment composition based on a sulfonamide agent, sulfadiazine 1% in combination with silver (*Sulfarginum or Sulfadiazine Silver* $C_{10}H_9AgN_4O_2S$) [6, 7] (Figure 1).



4-amino-N-pyrimidin-2-yl-benzenesulfonamide silver salt

Figure 1. Structure of sulfadiazine silver solt (lactam form)

Therefore, in addition to the biological properties of API, the clinical effect also depends on the method of administration, which is achieved by creating the appropriate form of the drug. Despite the apparent biocidal effect of the use of silver compounds, the description of the mechanisms of biological activity was first announced in the 1890s with the introduction of the term "oligodynamic effect", thanks to the work of Swiss-German scientist Carl Wilhelm von Negeli [8]. This means the toxic (from greek *oligos* — small, *dynamis* — force, "acting in small doses") effect of metal ions (Ag^+, Cu^{+2}, Au^{+3}) on prokaryotes and eukaryotes in relatively small amounts [9]. Later, when using radiolabeled sulfadiazine, it was shown that silver in the form of nitrates and salts with sulfadiazine forms a complex with DNA in vitro and has the highest degree of bacterial binding. The interaction between silver and DNA can be described as a process: there is a weak, reversible intercalative interaction between DNA and the sulfadiazine moiety, during which sodium sulfadiazine competes with Ag for binding to DNA and then a stronger, tightly bound complex is formed that involves coordination of the silver atom [10]. Additional studies have shown that resistant bacteria are those that are unable to bind Ag+ ions more strongly to release AgCl [11]. The antimicrobial activity of silver is based on the ability of Ag^+ ions to penetrate bacterial cell walls through pinocytosis, which leads to increased cellular oxidative stress followed by protein denaturation and growth inhibition [12]. Ionic silver also has the ability to bind to the microbial genome (DNA or RNA), which inhibits the replication of nucleic acids and prevents the reproduction of microorganisms.

At the beginning of the 20th century, innovative forms appeared in clinical practice, such as silver foil with silver nanoparticles (AgNPs), which proved their effectiveness in the treatment of burns and wound defects, including skin grafts. The use of silver foil not only reduced pain in patients, but also demonstrated pronounced bactericidal properties, which accelerated healing processes by stimulating the formation of granulation tissue. The emergence of new medical applications for silver is associated with the development of its unique forms, such as nanoparticles (AgNPs), and their integration into various materials, such as polymers and biocompatible matrices. One of the most important advantages of antimicrobial coatings is their ability to continuously disinfect a surface as long as it remains undamaged. Although this does not eliminate the need for regular sanitary measures, such coatings provide additional protection between hygiene procedures. For example, it has been found that pre-treated silver surfaces are able to effectively destroy fungi, mold and bacteria even after ten years of continuous use both at home and in medical institutions. Table 1 shows the forms and compounds of silver [13].

A surge of publications on nanoparticles, formed dispersions, and medical applications can be traced in international databases since 1995 [14]. During this period, the first publications appeared in the Russian-language scientific literature, including patents for inventions of methods for the treatment of purulent wounds using a pharmaceutical substance based on silver (d~nm) nanoparticles stabilized with polyvinylpyrrolidone (PVP) — poviargolum (Figure 2).

Table 1

Properties of silver atoms, ions and compounds

Form (ionic/neutral) of silver	Electronic formula (outer energy levels)	Compounds
Ag^0	$d^{10}s^1$	Ag nanodispersions
Ag^+	d^{10}	Ag_2O, $[Ag(OH)_2]^-$, $[Ag(H_2O)_4]^+$, AgF, AgCl, $AgNO_3$, Ag_2SO_4, Ag_2S, $Ag(CN)_2^-$
Ag^{+2}	d^9	AgF_2, $[Ag(C_5H_5N)_2]^+$, $AgSO_4$
Ag^{+3}	d^8	$[AgF_4]$–, $[AgF_6]^{3-}$, rare

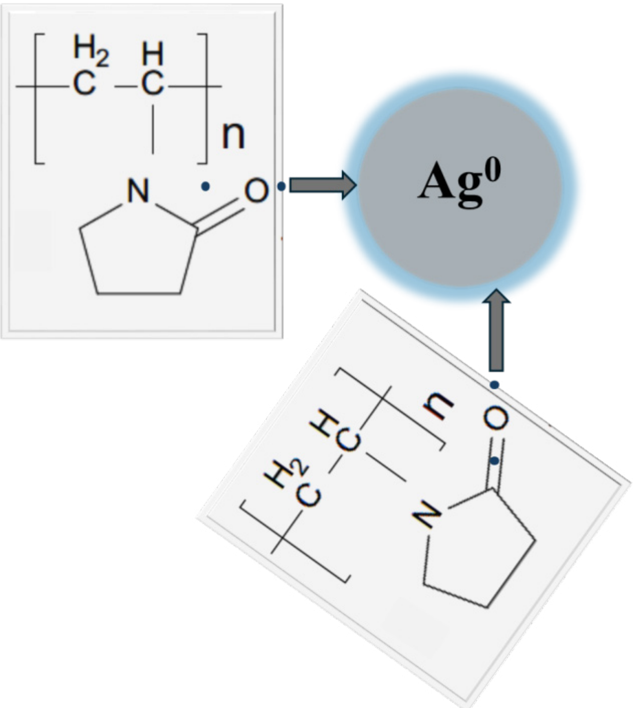


Figure 2. Binding of polyvinylpyrrolidone (PVP) with Ag nanoparticles

The synergistic effect of silver and PVP is directed against Gram-positive bacteria: *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*, resistant to carbapenems, aminoglycosides, polymyxins and tigecycline [15].

Structure and properties of silver nanoparticles

Silver nanoparticles (AgNPs) stand out among metallic nanoparticles for their unique physicochemical properties and biological effects, which makes them the subject of close study in the field of nanobiotechnology [16]. AgNPs particles exhibit various size-dependent properties in the range of 1–100 nm, where quantum phenomena are involved. A decrease in the size of nanoparticles contributes to a more pronounced manifestation of the quantum size effects (QZE), which manifests itself in the quantization of the energy of the excited state of charge carriers — the electron gas in the nanoparticle system and in the shift of the conduction band upward in energy levels (Figure 3).

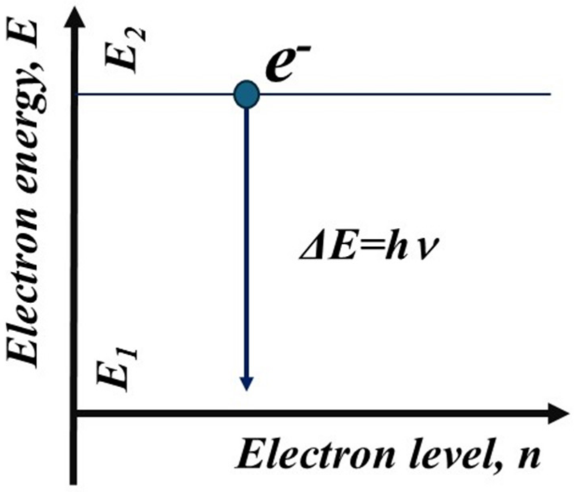


Figure 3. Discreteness of electron energy

The transition of an electron from one stationary state (E_2) to another (E_1) is accompanied by the emission of a quantum of light with a frequency:

$$\nu = \Delta E / h, \quad (1)$$

where ΔE is the energy difference between the initial and final states of the electron, and h is Planck's constant.

The quantum structures of the material are characterized by unique optical and magnetic properties that differ from those of physical structures in which particles have a size exceeding the de Broglie wavelength (macroscopic bodies). Fluorophores based on quantum heterostructures are actively used in medicine and biology, for example, in tagging and imaging systems for biological objects, including in the detection and control of tumor cells [17]. These properties are significantly influenced by factors such as surface area, surface charge, morphological diversity (for example, rods, triangles, color-like structures, spheres), as well as size variations from 1 to 100 nm. For example, spherical AgNPs with smaller dimensions (d~1–20 nm) exhibit excellent antibacterial activity due to their increased reactivity. In contrast, larger spherical particles or particles with similar sizes but different shapes, such as triangular nanoparticles with sharp edges, exhibit different efficiencies, with triangular shapes often outperforming spherical ones due to their reactive edge structures (Figure 4).

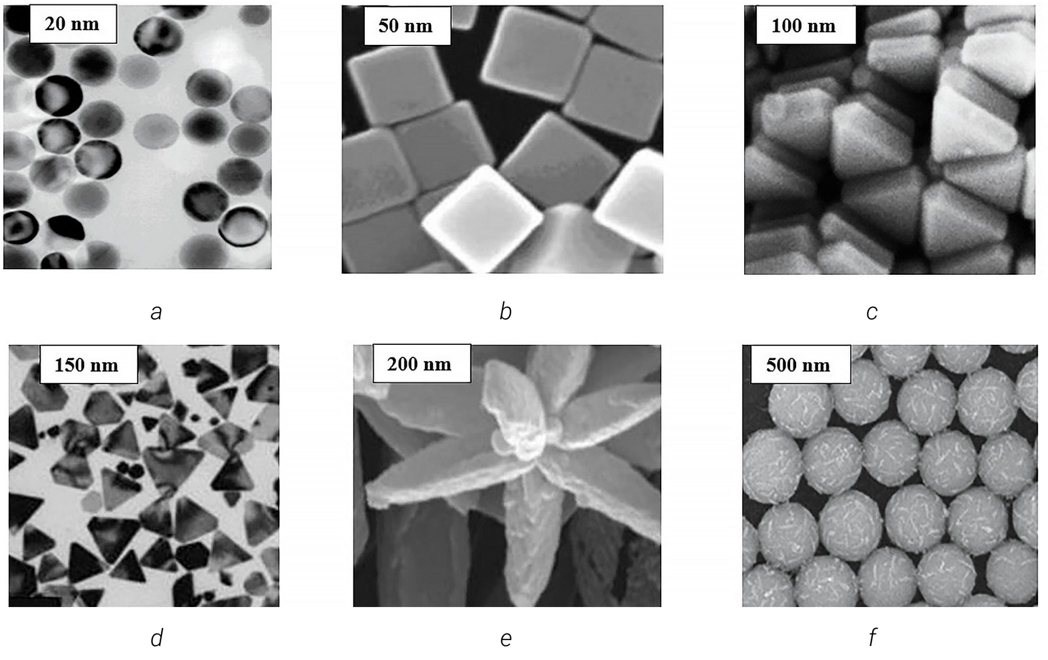


Figure 4. Different shapes of Ag nanoparticles depending on size:
(*a*) sphere, (*b*) a nanocubes, (*c*) pyramids, (*d*) prisms, (*e*) flower, (*f*) ellipse [21]

The stated differences in the antibacterial effectiveness of nanoobjects can be explained by the anisotropic shape of some silver nanoparticles, which differ in the dense arrangement of atoms on the surface, which enhances the effect on their surface, enhancing the biocidal effect [18]. Experimental data show that quasi-spherical AgNPs (~20 nm in diameter) and spherical particles with a smaller diameter (~9 nm) exhibit pronounced antimicrobial effects. In addition, AgNPs synthesized in a phosphate-salt buffer in the size range of 5–20 nm exhibit significant antiviral activity [19]. Thus, the functional versatility of AgNPs in medicine is closely related to their size, shape, and surface morphology. This diversity highlights the need for ongoing research to optimize their design and application in this dynamic field of research [20].

The growth of nanoparticles during solution-phase synthesis proceeds in stages: nucleation — seeding — growth. One can control the shape of the synthesized nanoparticles by tuning the thermodynamics and kinetics at each stage of their solution-phase synthesis [21]. Thus, the shape and size of the resulting silver nanoparticles depend on experimental conditions such as: synthesis temperature, concentration of the starting materials and stabilizer, pH of the solution, as well as the approach used (“bottom-up” or “top-down”).

Mechanism of action and targets

Silver nanoparticles (AgNPs) exhibit exceptional quantum dimensional properties due to their pronounced surface area, ability to interact with microbial biomolecules, penetrate cells, generate reactive oxygen species (ROS) and free radicals, and interact with microbial signaling molecules [22, 23]. Modification of the surface of AgNPs can enhance their oligodynamic effects (in small doses), improving stability and physico-chemical characteristics. Metallic silver ions become biologically active as a result of reduction under the action of oxidizing agents — sodium citrate, glucose, etc. [24]. The restored form binds to the membranes of bacterial cells, causing structural damage and morphological changes. Unlike other metal nanoparticles, AgNPs minimize toxic effects. The authors [25] cite research results confirming significant changes in antibiotic sensitivity and the ability to form bacteria biofilms (multicellular-like behaviors or forms) observed during prolonged exposure of pathogens causing diseases of the genitourinary system to combinations of chemotherapeutic antibacterial agents and AgNPs.

For example, copper nanoparticles (CuNPs) exhibit a more pronounced antibacterial effect, but their cellular toxicity limits their practical use. Particles CuNPs destroy bacterial membranes and interfere with electron transfer during photocatalysis, but their harsh effect contrasts with the balanced effectiveness of silver. The paper [26] presents the results of a study of the wound-healing activity of methylcellulose-based ointments with different concentrations of copper nanoparticles measuring 103 nm (71% crystalline copper). It was found that an ointment containing copper nanoparticles at a concentration of 0.002% achieves the optimal effect on wound healing.

The proposed mechanisms of action of AgNPs include adhesion to cell walls/membranes, intracellular penetration; disruption of organelles and induction of oxidative stress and modulation of signaling pathways (Figure 5).

Silver nanoparticles are able to firmly attach and accumulate on the surface of gram-negative bacterial cells, as well as penetrate into microbial cells through pores, which are water-filled channels located in the outer membrane of gram-negative bacteria [28]. Porins play a key role in the passive movement of hydrophilic molecules of various sizes and charges through the membrane. On the contrary, the thicker cell wall of Gram-positive bacteria can prevent the penetration of silver ions into the cytoplasm [29]. Lipopolysaccharides present in the cell walls of gram-negative bacteria probably help maintain their structural integrity, but also make them more vulnerable to silver nanoparticles. The negative charge of lipopolysaccharides further promotes the adhesion of nanoparticles. Some studies show that silver nanoparticles can adhere to the surface of bacteria, altering the properties of membranes and potentially causing damage to bacterial DNA [30].

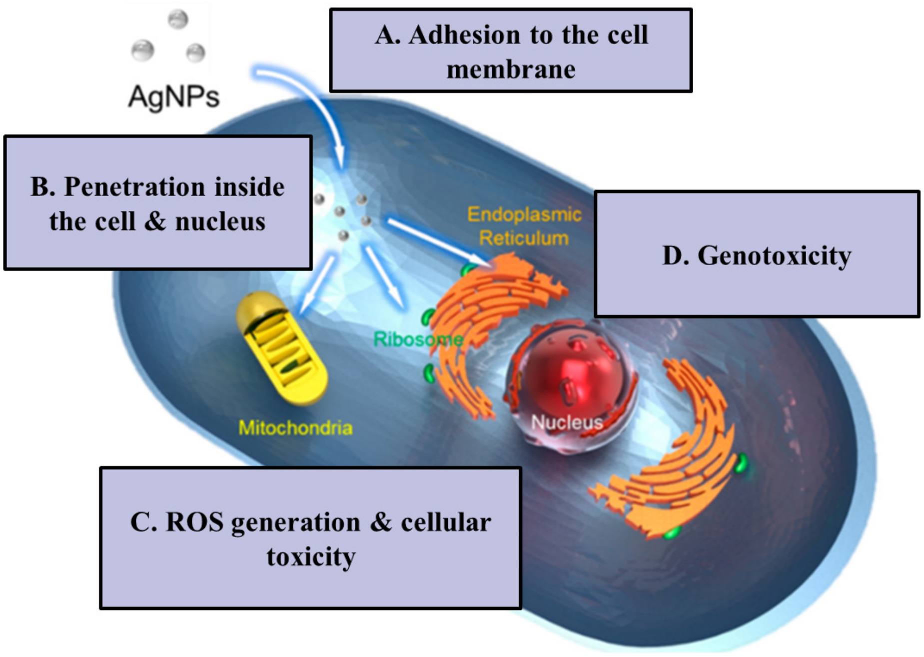


Figure 5. Ways of biocidal action of AgNPs: (A) alters membrane structure & permeability, leakage of cellular content & ATP, (B) destabilize and denature proteins, interact with DNA, (C) oxidize proteins & lipids, mitochondrial dysfunction, ribosome destabilization, (D) DNA base damages, inhibition of replication and transcription [27]

Since the attachment of bacteria to the surface is the first step in the development of biofilms (polycellular forms), silver nanoparticles binding to the cell surface can hinder this process. This aspect is of great practical importance in the fight against pathogens, indicating that silver nanoparticles can be useful not only for preventing infections, but also for combating antibiotic-resistant strains [31, 32].

Synthesis methods

To obtain nanoparticles with the claimed properties, a carefully controlled synthesis process is necessary. Since the advent of nanotechnology, many methods of producing silver nanoparticles have been developed. In general, these approaches are divided into two main categories: “top-down” and “bottom-up” (Figure 6).

The top-down method involves crushing massive materials into metal nanoparticles using various physical methods such as mechanical crushing, grinding, as well as electrical methods such as electric discharge and laser ablation. Thermal methods, including vapor condensation, also play an important role. Physical methods usually produce nanoparticles of high purity and uniform size. Although such processes do not require potentially dangerous chemicals or stabilizers, they require complex and expensive equipment, as well as significant energy consumption.

In contrast, the bottom-up method is based on the assembly of nanoparticles from molecular building blocks through the processes of growth and nucleation. Chemical and biological synthesis are widely used in this approach. Chemical synthesis can be accelerated and optimized using various energy sources — visible or ultraviolet light, electricity, microwaves, or ultrasound — which makes it possible to quickly obtain nanoparticles of various shapes [34]. However, chemical synthesis can be accompanied by risks associated with toxic by-products, which limits its use for medical and pharmaceutical purposes.

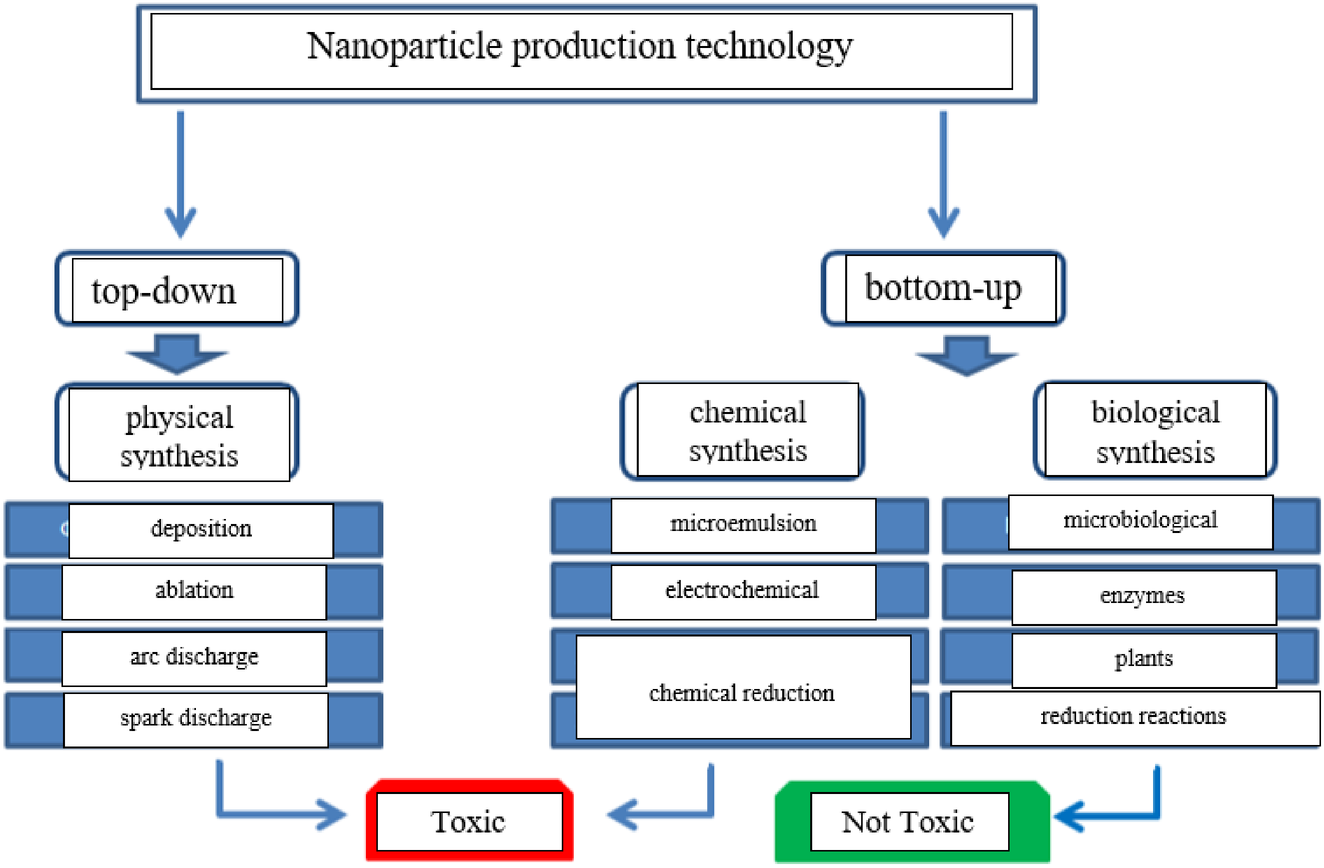


Figure 6. The main approaches to obtaining nanoparticles [33]

Biological synthesis is attracting increasing attention due to its environmental safety and sustainability. It uses biologically active compounds such as alcohols, flavonoids, and phenolic compounds as reducing agents [35].

Traditional methods of nanoparticle synthesis can be expensive and pose risks to human health and the environment. To overcome these disadvantages, researchers are actively exploring so-called “green” methods using natural materials and their derivatives. Biological synthesis can be divided into methods using fungi, yeasts (eukaryotes), bacteria, and actinomycetes (prokaryotes), as well as approaches based on plants, plant extracts, and even viruses as templates [36, 37].

The chemical synthesis of silver nanoparticles consists in the reduction of silver salts in water or organic solvents. This process is based on three key components: reducing agents, metal precursors, and stabilizing or coating organic polymers. The choice of reducing agents and coatings is extremely important, since they can significantly affect the physico-chemical properties of the released nanoparticles [38, 39]. Despite these disadvantages, chemical methods provide high yield, excellent reproducibility, and lower cost compared to physical methods.

Physical synthesis, as a top-down method, uses physical effects such as electromagnetic radiation, plasma, and thermal effects. This method allows nanoparticles to be evenly distributed on thin films without contamination with solvents [40]. Vapor condensation and laser ablation are among the most common physico-chemical methods. Vapor condensation is a two — stage process involving evaporation and condensation in a specialized tubular furnace at normal atmospheric pressure; the size of the resulting nanoparticles varies from 3 to 50 nm [41]. However, physical synthesis is energy-intensive and time-consuming, which can lead to an increase in ambient temperature.

Nanodispersions from 10 to 30 nm with a given morphology and properties can be obtained by laser ablation in a liquid using an environmentally friendly and affordable approach [42].

The choice of a method for synthesizing silver nanoparticles is a critically important decision that depends on the required characteristics of the final product and its field of application. Each top-down or bottom-up approach has its advantages and limitations, which requires careful analysis in terms of environmental safety, economic efficiency, and technological feasibility.

The latest application

More and more studies indicate the occurrence and stable retention of pathogens (bacteria d>5 mcm, viruses d~20–200 nm, protein infectious particles of prions d<5 nm, fungi, yeast and protozoa d>200 mcm) in patient care in clinical settings, which can cause infection of hospital patients and cross-contamination. The infections between them are nosocomial infections [43]. Widespread disinfectants alone can stimulate the development of resistance. For example, quaternary ammonium compounds (QACs) have long been considered an effective class of disinfectants that are immune to bacterial resistance. However, methicillin-resistant staphylococcus aureus (MRSA) isolates show an increase of about 30% in their resistance genes. Disinfection of rooms by exposure to high-intensity UV light can be effective in destroying most microorganisms. However, this measure requires the complete emptying of the room by both staff and patients during the process in accordance with safety requirements [44]. As a result, the development of new materials with surface antimicrobial properties based on metallic silver nanoparticles is extremely promising for medical applications.

Silver has been used in medicine for many decades, and its antimicrobial activity is recognized as one of the first effective disinfection technologies [45]. Silver nanoparticles are widely used in dressings — numerous studies confirm that they significantly accelerate wound healing and at the same time prevent the development of bacterial infections. As already noted, the ability to bind to various molecular targets in microbial cells provides silver nanoparticles with a prolonged antibacterial effect. For example, AgNPs incorporated into the bacterial cellulose matrix exhibit pronounced bactericidal activity against gram-negative bacteria such as *Escherichia coli*, while not having a toxic effect on epidermal cells, which makes them ideal for medical use [46].

In modern experimental medicine, the possibility of synergetic use of silver nanoparticles as powerful antibacterial agents in combination with various antibiotics is actively being investigated [47]. However, it is worth noting that this synergy is not observed for all classes of antibiotics, which requires further study of the mechanisms of their interaction.

Despite all the attractive advantages of silver-based drugs for medicine, it should be remembered their possible toxic effects and contraindications to use. Considered [48], that the toxicity of silver is a rare diagnosis, usually manifested by skin manifestations in the form of argiria disease (greek. ἄργυρος silver). Long-term deposition of silver compounds (silver dust, colloidal silver or silver nanoparticles) in a dose of 70 to 1500 mg Ag/kg body weight leads to irreversible strong skin pigmentation. An *in vivo* study using radiolabelled silver showed the onset of accumulation effect 2–8 hours after exposure, with a half-life period of 2 weeks. Oral absorption through the gastrointestinal tract may vary depending on other food components that potentially affect the bioavailability of silver; absorption is up to 10% [49]. Metal silver presents minimal health risk — according to the American Conference of Governmental Industrial Hygienists (ACGIH), the threshold limit for metallic silver is 0.1 mg/m^3, while soluble silver compounds — an order of magnitude less than 0.01 mg/m^3. A single lethal dose of silver nitrate is estimated to be about 10 g, an intravenous dose of about 50 mg of silver causes necrosis of bone marrow, kidneys and liver, bleeding and swelling of the lungs [50, 51]. However, such cases are known only among suicidal or other preventive actions.

In addition to medical purposes, silver nanoparticles are used as effective heterogeneous catalysts that are actively used to reduce halogenated organic pollutants. Their unique catalytic properties also enhance the bleaching ability of organic dyes. Tubular silver nanoparticles have high catalytic activity, which expands their use as effective catalysts for the tasks of green chemistry and environmental restoration [52–54]. The particles AgNPs are widely used not only in medicine, but also in other scientific fields due to their unique physico-chemical properties — high conductivity and photocatalytic activity. One of the key areas of their use is electronics, where AgNPs are used as conductive materials to create innovative flexible electronics, sensors and solar panels, opening up new opportunities for energy-saving technologies.

Conclusion

The metal and oxide nanoparticles have proven to be an original potential approach for applications in a wide variety of fields, from medicine to physics and chemistry. This was made possible by their pronounced antimicrobial properties and high biocompatibility. Silver, known to mankind for centuries, has become an important element in various fields of culture and medicine, primarily due to its ability to effectively resist microorganisms. Numerous studies confirm that silver nanoparticles significantly accelerate wound healing and prevent bacterial infection due to the high reactivity of the surface and the ability to interact with cellular structures of pathogens. The introduction of silver nanoparticles into medical devices and technologies is a breakthrough area that requires special attention and further study. The combination of silver nanoparticles with traditional antibiotics opens up new horizons in the treatment of infectious diseases, although additional research is needed to optimize such synergistic interactions. Given the growing interest from the scientific community and industry, silver nanoparticles remain an important research object, confirming their significant potential for developing effective solutions that promote sustainable development in a wide variety of industries.

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Серебряные наночастицы: известные и неизвестные факты в обзоре свойств, механизмов действия и применения в медицине

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Аннотация. *Актуальность.* Обзор посвящен современному состоянию дел в области использования нанообъектов в медицине и фармации. Актуальность этой темы обусловлена уникальными свойствами наноматериалов, которые демонстрируют различные размерные зависимости в диапазоне 1–100 нм, где вступают в игру квантовые явления. *Цель* данной работы — обозначить основные стратегии современной науки по разработке и успешной стабилизации наночастиц, предназначенных как для диагностики, так и для лечения патологических заболеваний. Наноструктурировнаные гетерогенные матрицы, содержащие высокодисперсные частицы с размером, соответствующим длине волны де Бройля, открывают многообещающие возможности для их использования в различных не только в научных, но клинических исследованиях. Разнообразие природы, морфологии, размеров и свойств наночастиц, применяемых в медицине для диагностики и терапии заболеваний бактериальной, вирусной и злокачественной этиологии, стимулирует интерес к частицам, проявляющим квантово-размерный эффект. Квантовые структуры нанообъектов характеризуются уникальными оптическими и магнитными свойствами, отличными от свойств физических структур, в которых частицы имеют размер, превышающий 100 нм (макроскопические тела). Флуорофоры на основе квантовых гетероструктур активно применяются в медицине, биологии например, в системах мечения и визуализации биологических объектов в том числе при обнаружении и борьбе с опухолевыми клетками. *Выводы.* В данном обзоре описаны современные подходы к синтезу и функционализации наночастиц на основе принципов «сверху вниз» и «снизу вверх», а также потенциальные механизмы действия наночастиц в зависимости от их морфологии, размера и ионной (атомной) формы. Обсуждаются уникальные физико-химические свойства наночастиц, обусловленные квантовыми эффектами размера. В статье описываются различные формы наночастиц, механизмы действия, молекулярные мишени, а также методы синтеза и применения. Эти аспекты имеют решающее значение для понимания роли наночастиц в борьбе с инфекциями и разработке новых терапевтических подходов, обеспечивая их постоянную актуальность в современной науке и медицине.

Ключевые слова: наночастицы серебра, биоцидные свойства, антимикробные поверхности, квантово-размерный эффект, олигодинамический эффект, «зеленая» химия

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